IN THE NAME OF GOD

دانشگاه علوم پزشکی قزوین

Review Physicochemical Characteristics Of Nanobody-HER2 Complex

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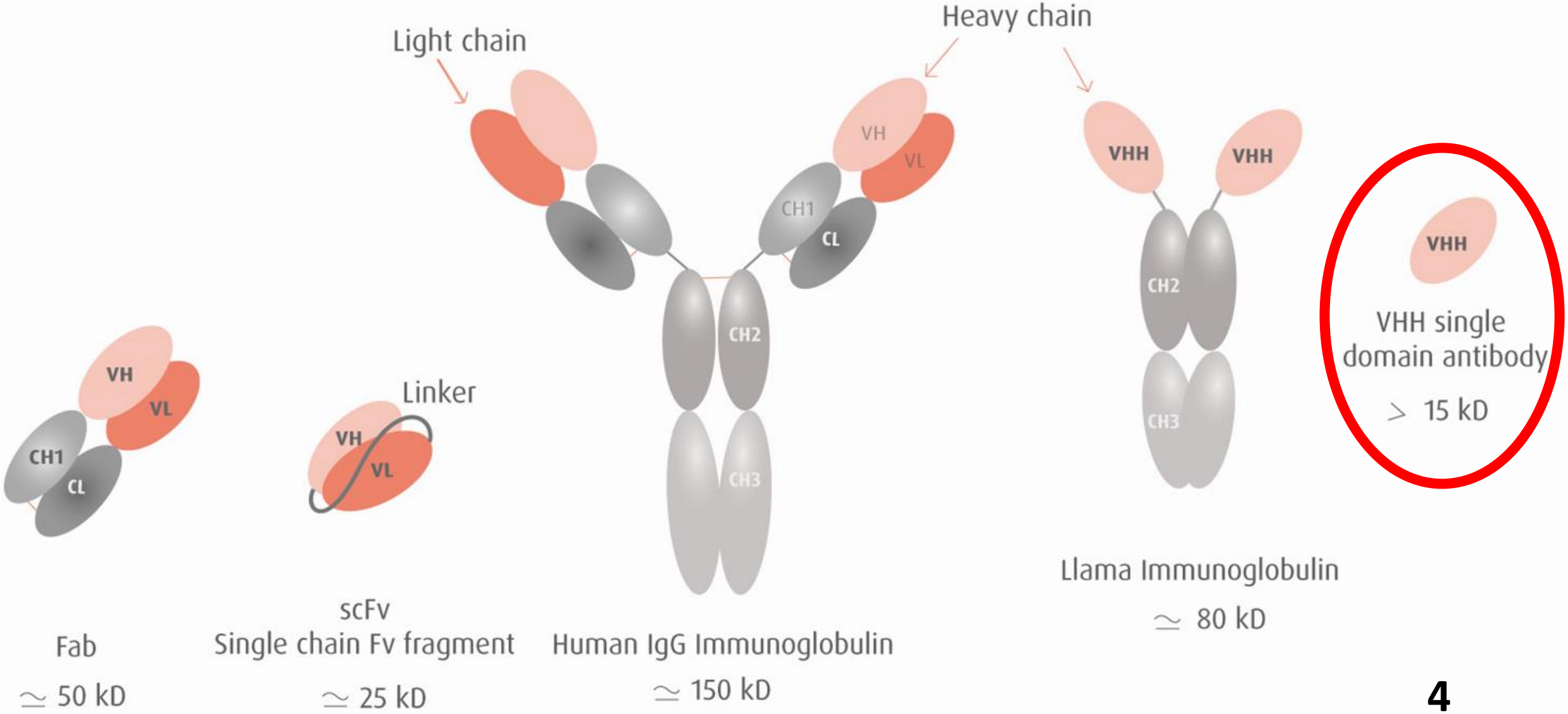
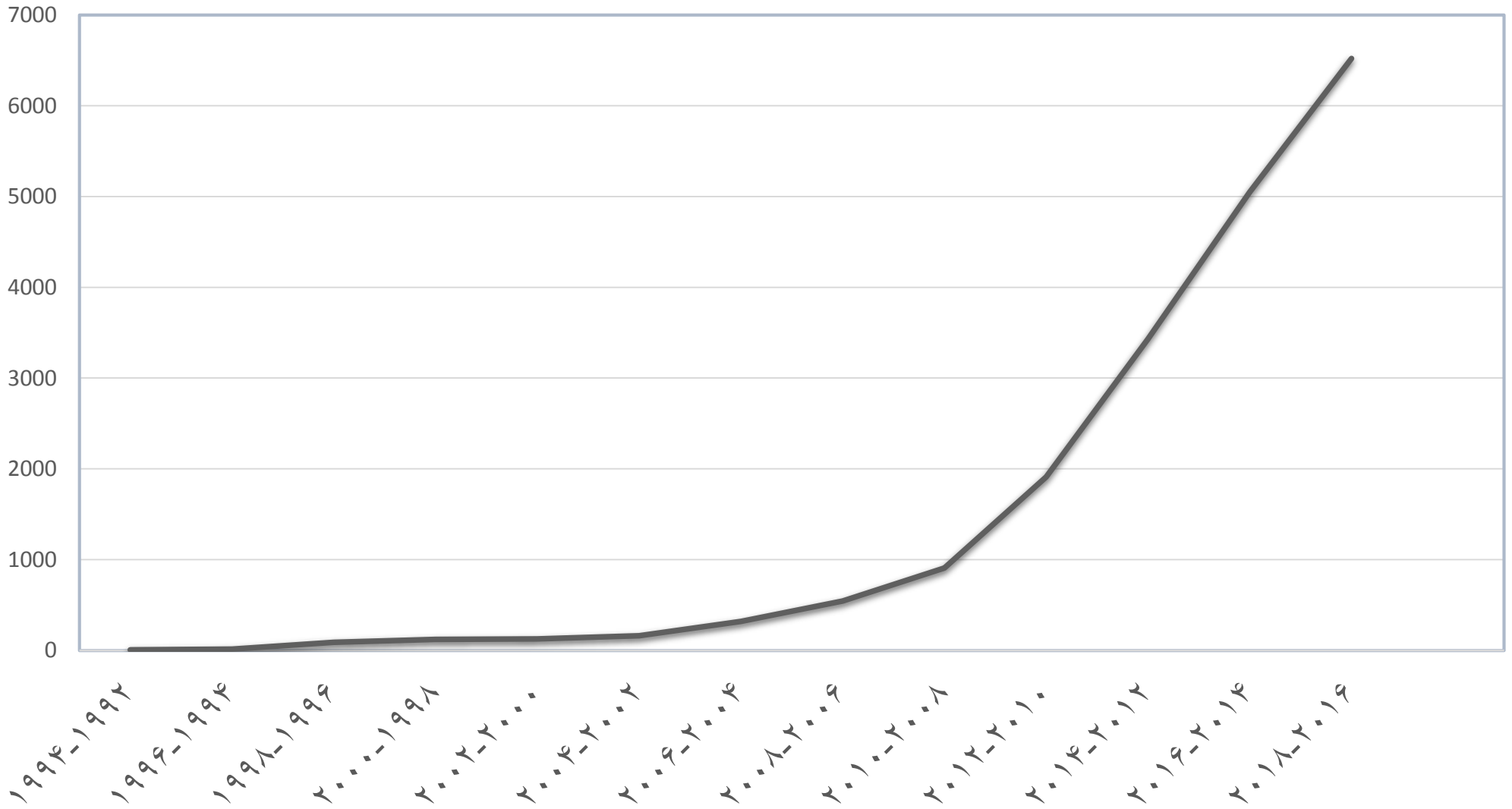
List of content

Formats Derived From Antibodies



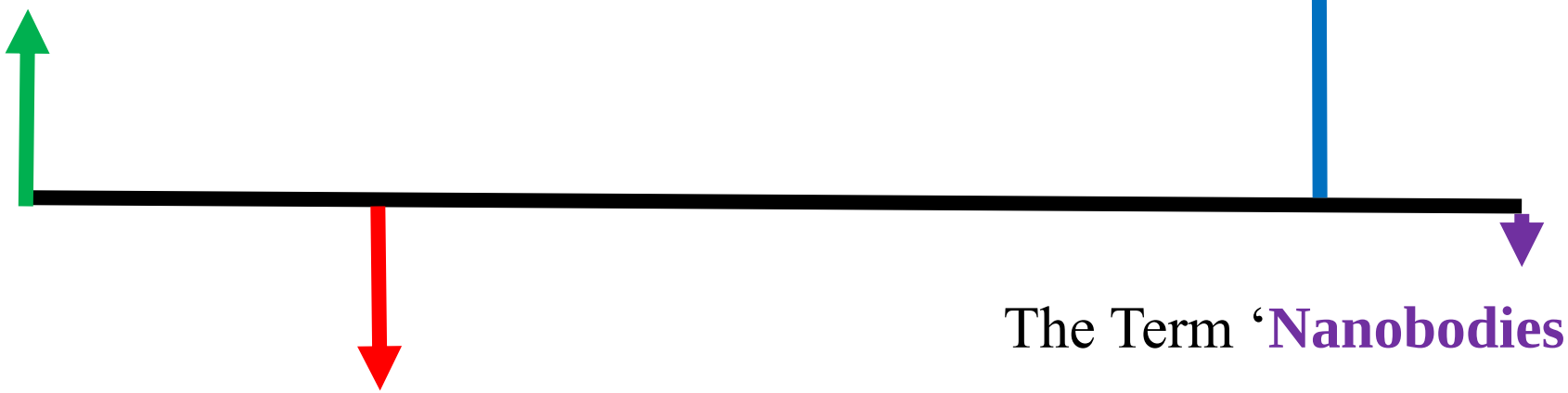
Research Trend Of Nanobody



Timeline Of Nanobody

Muyldermans & co-workersIn **1994**, **sequence**

Hamers-casterman In **1993 ,** In Brussels (1)

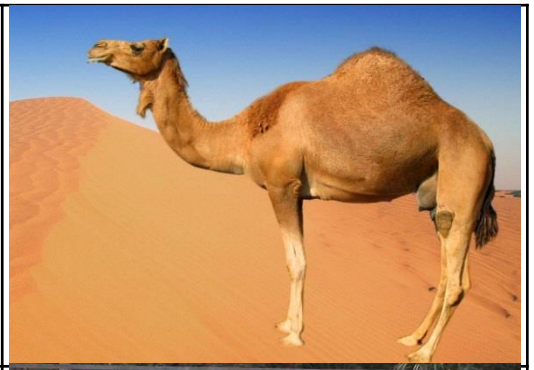
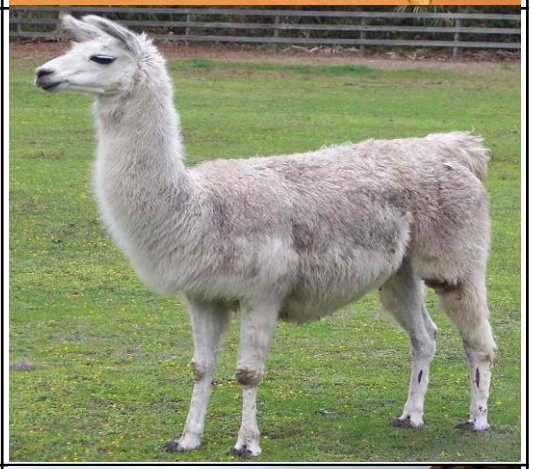


The Term '**Nanobodies**' (Ablynx®)

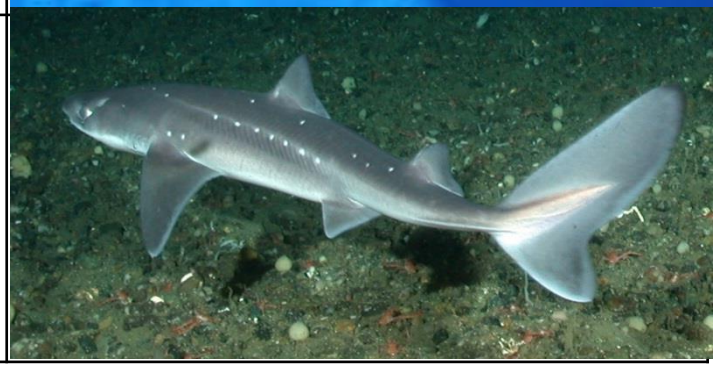
Separated from **serum *via* Protein A and Protein**

G chromatography 1993

Nb Species Producer

In camels (*Camelus dromedarius* & *Camelus bactrianus*)	
Llama (*Lama glama* & *Lama guanicoe*)	
Vicugna (*Vicugna vicugna* & *Vicugna pacos*)	

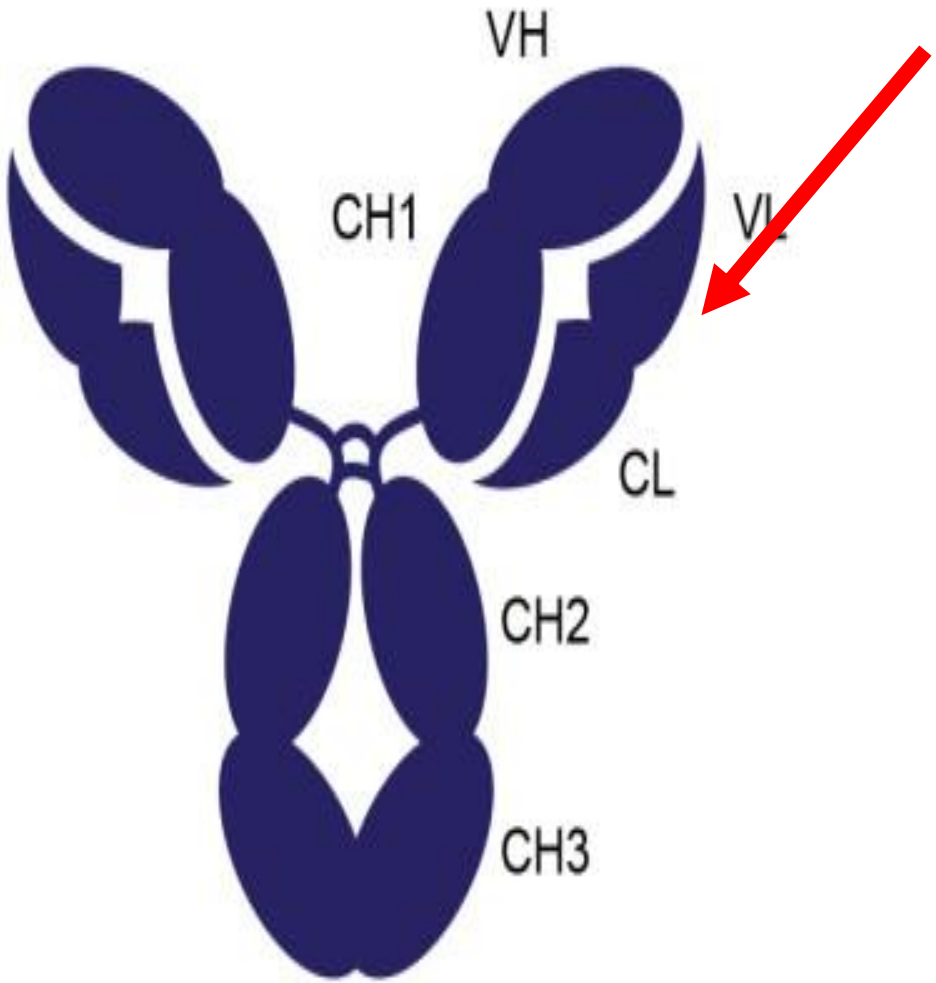
Nb Species Producer

Nurse shark (*Ginglymostoma cirratum*)	
Wobbegong (*Orectolobus maculates*)	
Dogfish (*Squalus acanthias* & *Mustelus canis*) sharks	

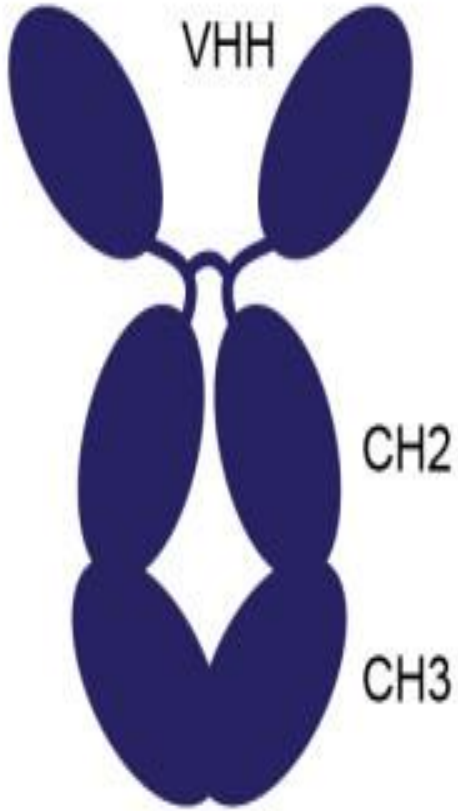
Reagent	Pros	Cons
Antibodies	1. **Bi**valent 2. **Poly**clonal 3. **Labeled** With **Multiple Dyes** 4. **Commercially** Available	1. **Not Recombinant**; Require Animal **Sacrifice** 2. **Poor** Tissue Penetration 3. **Less Resolution** Due To Larger Label Displacement 4. Require Separate Incubation Of **1° & 2°** 5. Must Use Different Species And/Or **Different IgG** Subclasses Of 1° For **Multi-color Staining** 6. Can Be **Expensive**
Nanobodies	1. **Recombinant**; do not require animal sacrifice 2. **Good** tissue penetration 3. Can be labeled with multiple dyes 4. **Greater resolution** due to lower label displacement 5. **2° incubation** can be skipped by pre-binding to 1°	1. **M**onovalent 2. **Mono**clonal 3. Only **Anti- Rabbit** And **-Mouse** Versions Are Currently Available 4. **Not Commercially** Available

Structure & Characteristics

Key features	Key benefits
1. Small size (~**15 kDa**) 2. Not Contain A **Light Chain & CH1** Domain	Size **3*3*4 nm (Penetrate into per meabilized cell)**
1. Four Conserved regions (**FRs**) **2. Three** Connecting regions **CDRs** 3. Nine β-strands	1. Easily produced in **E. coli** or **yeast cells** **2. Humanize** By Modifying Few Residues In FR2 without altering properties(2)
1. Very **Soluble** & Highly **Stable** 2. High **Specificity** & Affinity For **Antigen & Thermal** & Conformational **Stability** 3. Homology with **human VH ,** low immunogenicity	1. Bind a wide range of epitopes with affinities in the **nm ,pm** range 2. Resistant to the acidic environment of the stomach **3. low aggregation (**more **hydrophilic ,** framework 2 **mutations**)



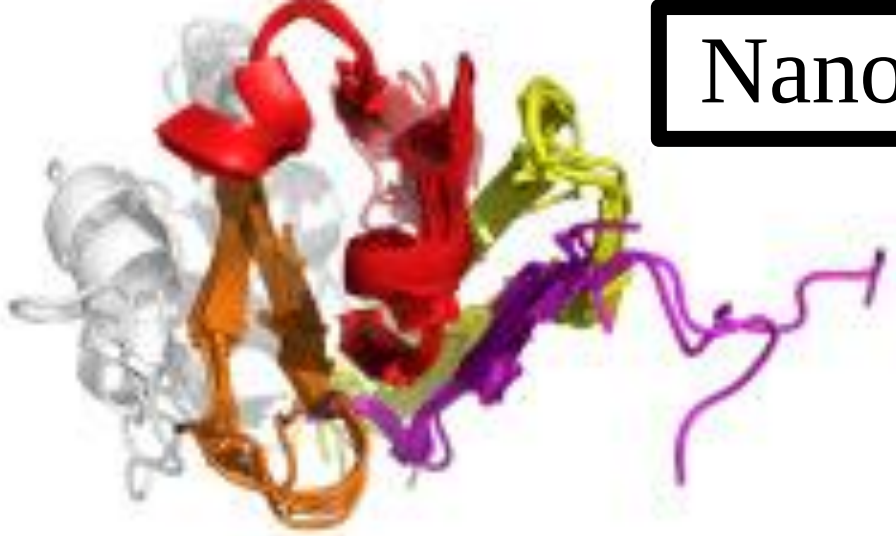
IgG

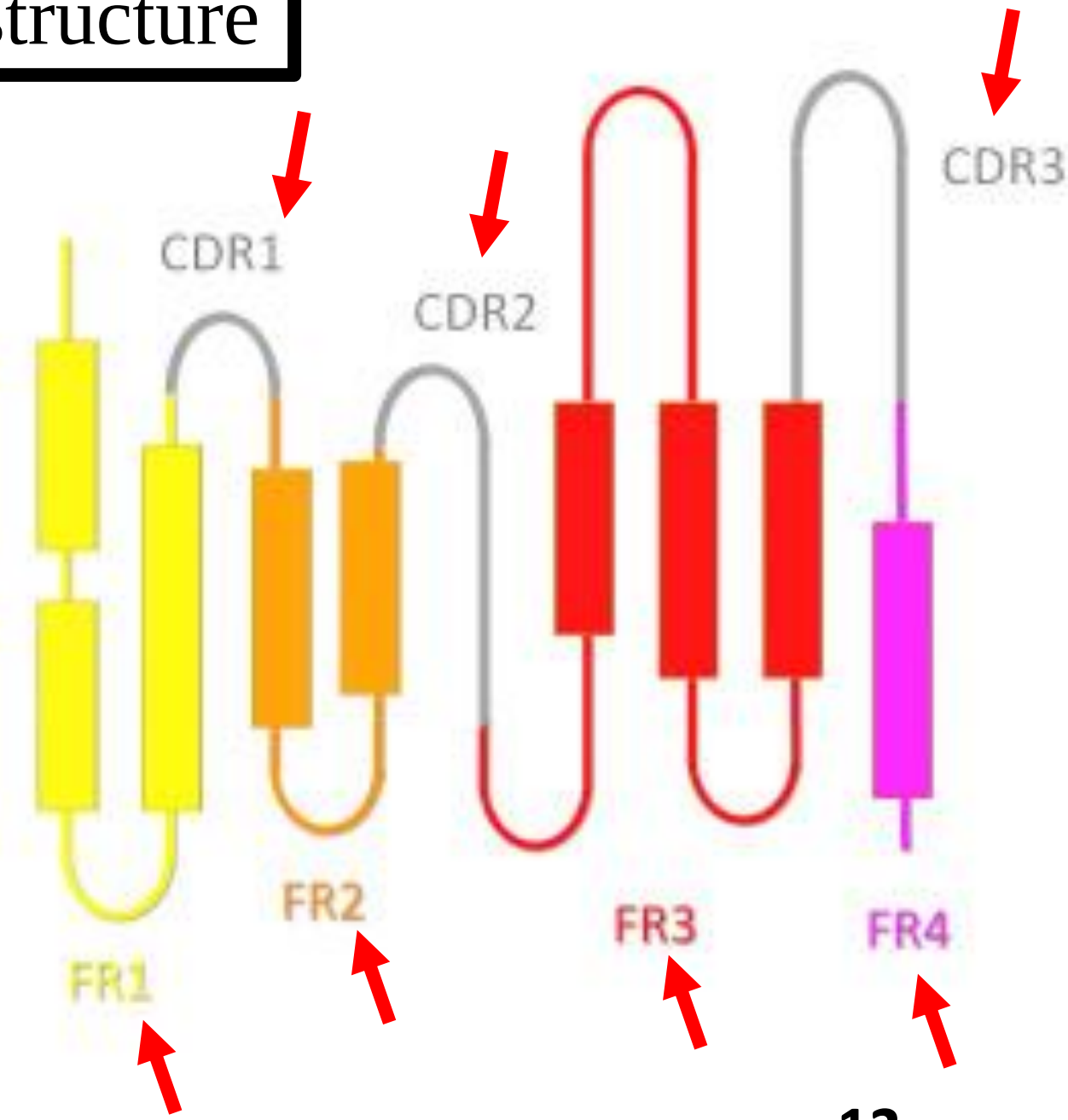


Camelid Ig

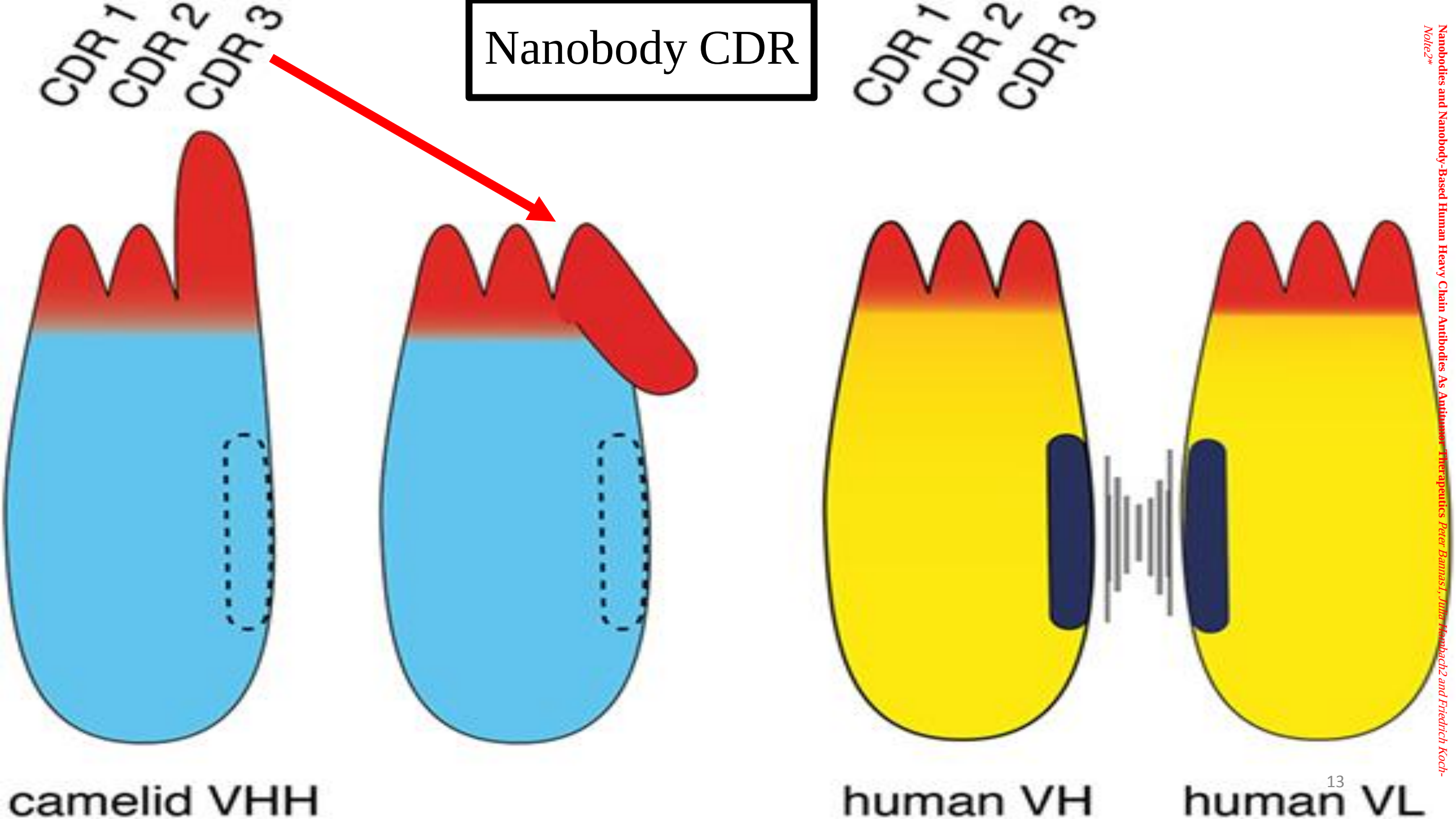
VHH

Nanobody structure





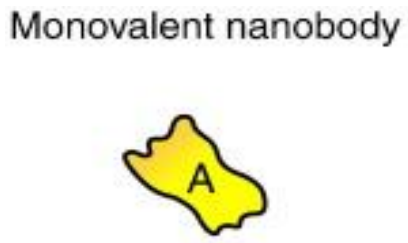
Nanobody CDR

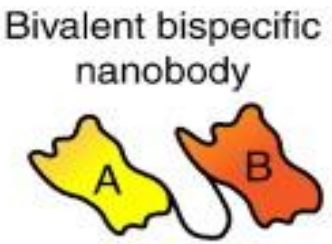


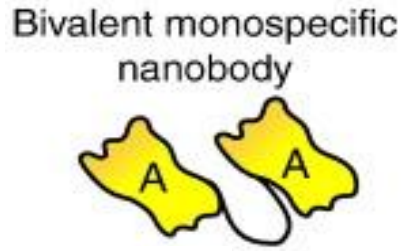
Nanobodies and Nanobody-Based Human Heavy Chain Antibodies As Antitumor Therapeutics *Peter Bannas1, Julia Hambach2 and Friedrich Koch-Nolte2**

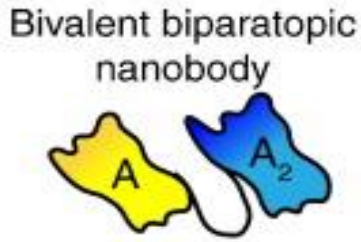
Characteristics

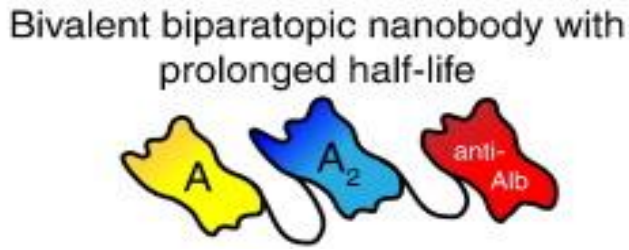
1. CDR3 human **VH is 13 aa** , Dromedaries **18 aa**
2. 50 file pdb database
3. Bivalent ,trivalent , bispecific ,biparatopic

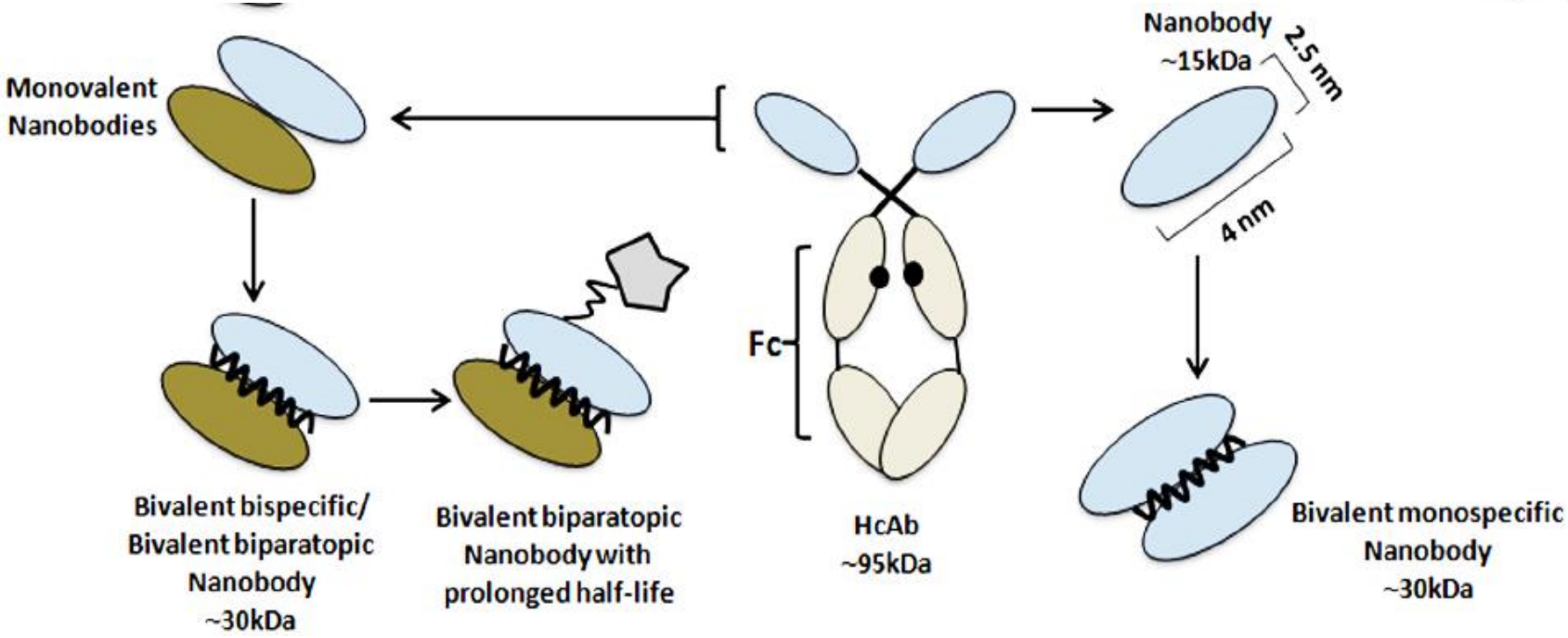




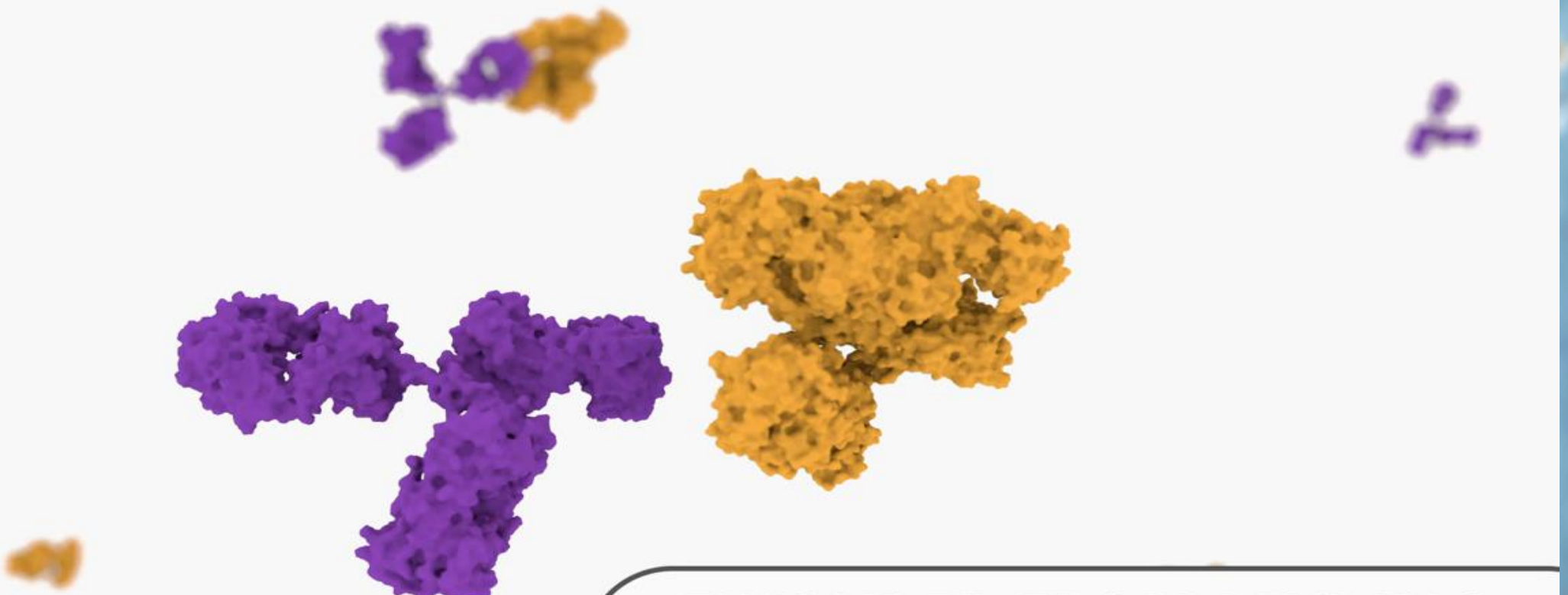


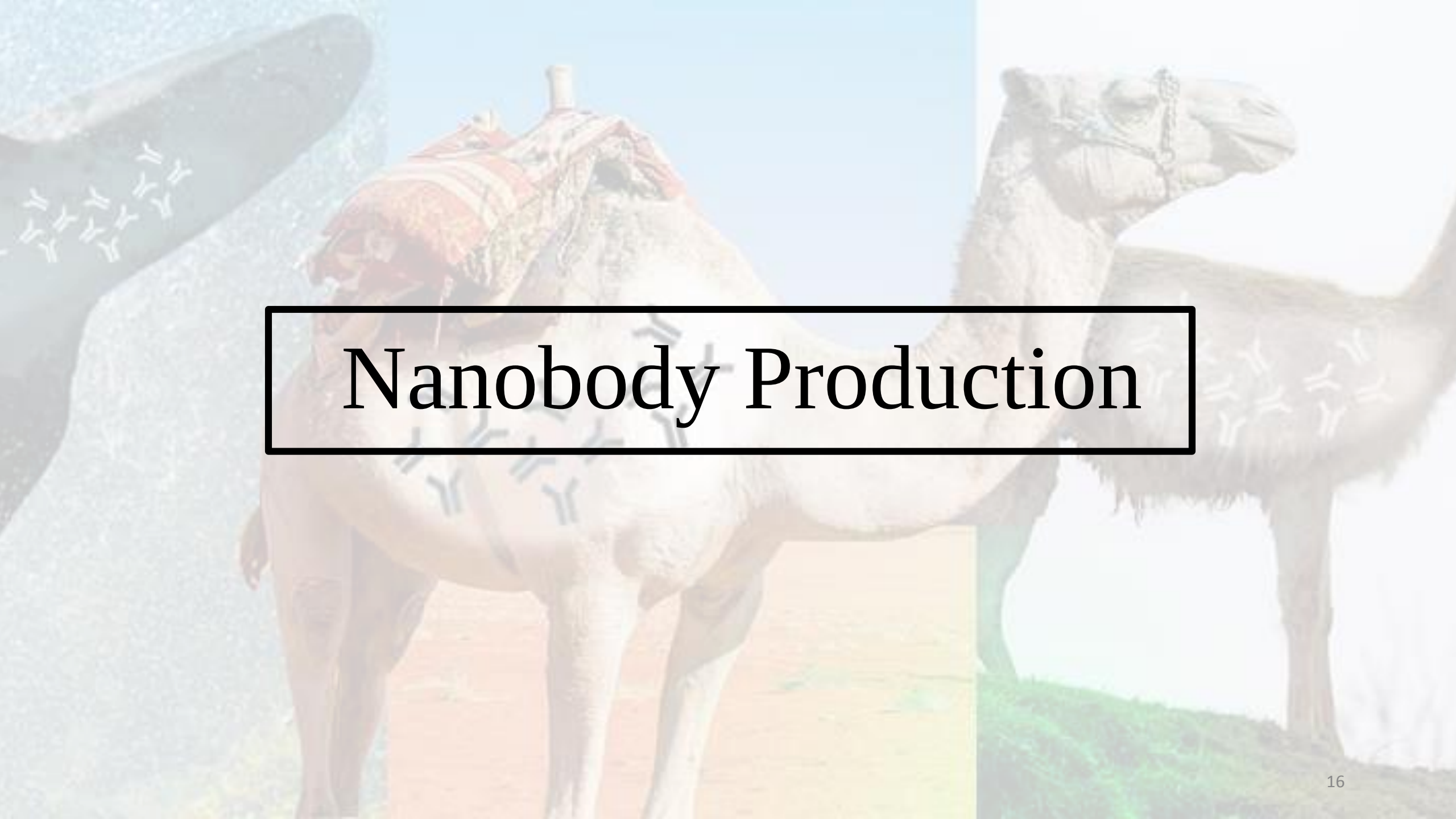






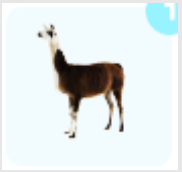
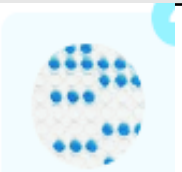
Nanobody

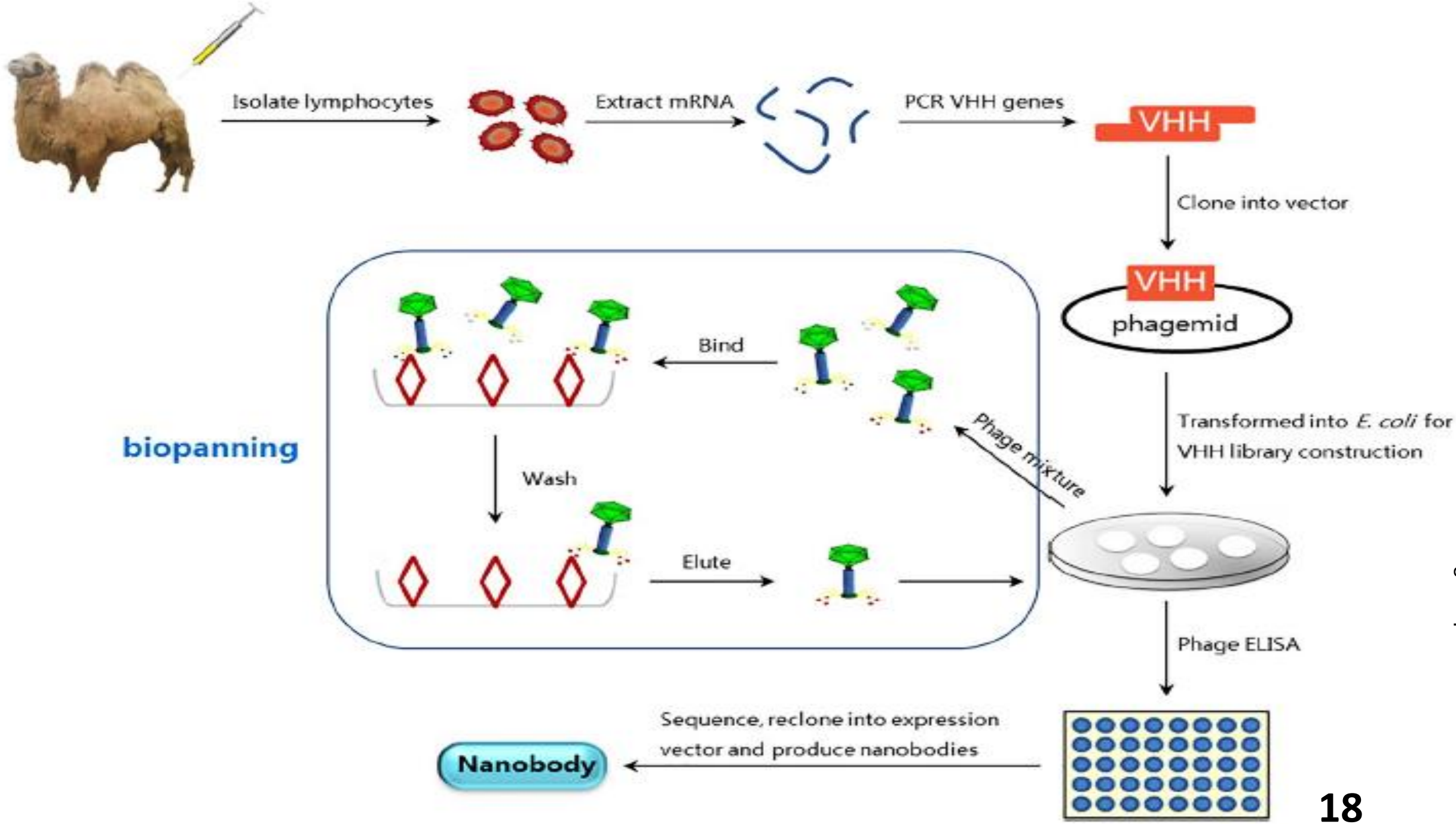




Nanobody Production

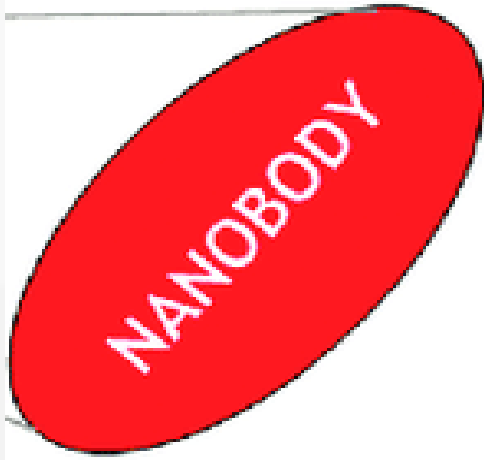
Nanobody Production

phase	Procedure	Description
I	Immunization 	Immunization of one Llama on standard protocol ELISA to assess the titer
II	Plasma Cell Isolation 	SPIN® technology isolates plasma cells
III	Single Domain Library Construction 	Single domain antibody genes are amplified to construct small libraries
IV	Expression and Screening 	ELISA or functional assays make antibodies suitable for downstream work.
V	Production 	Efficient HEK293 or CHO cell expression


Isolate lymphocytes
Extract mRNA
PCR VHH genes
VHH
Clone into vector
VHH
phagemid
Transformed into E. coli for
VHH library construction
biopanning
Bind
Wash
Elute
Phage mixture
Phage ELISA
Sequence, reclone into expression
vector and produce nanobodies
Nanobody

Nanobody

The way in which we produce



Nanobody Application

Nanobody Application

Non-invasive In Vivo Imaging

1. **Nanobody For Molecular Imaging Of Cancer**
    1. **In Nuclear Imaging**
    2. **Nanobody-targeted Ultrasound**
    3. **In Optical Imaging**

Therapeutic Nanobodies Directed Against Extracellular Targets

1. **Nanobody-mediated Drug Delivery**

Diseases Treatment :

1. Neurological
2. Inflammatory
3. Oncology

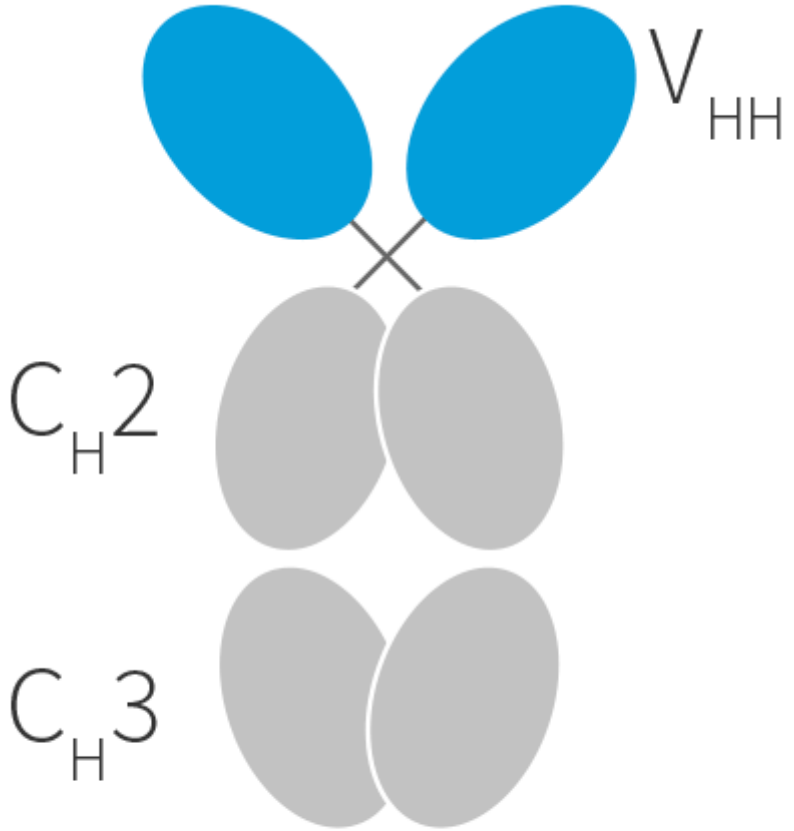
Linked To Enzymes Or Fluorescent Proteins

Antigen-targeting Molecules Inside Cells

2. Nanobodies Against Intracellular Targets: Tools To Identify New

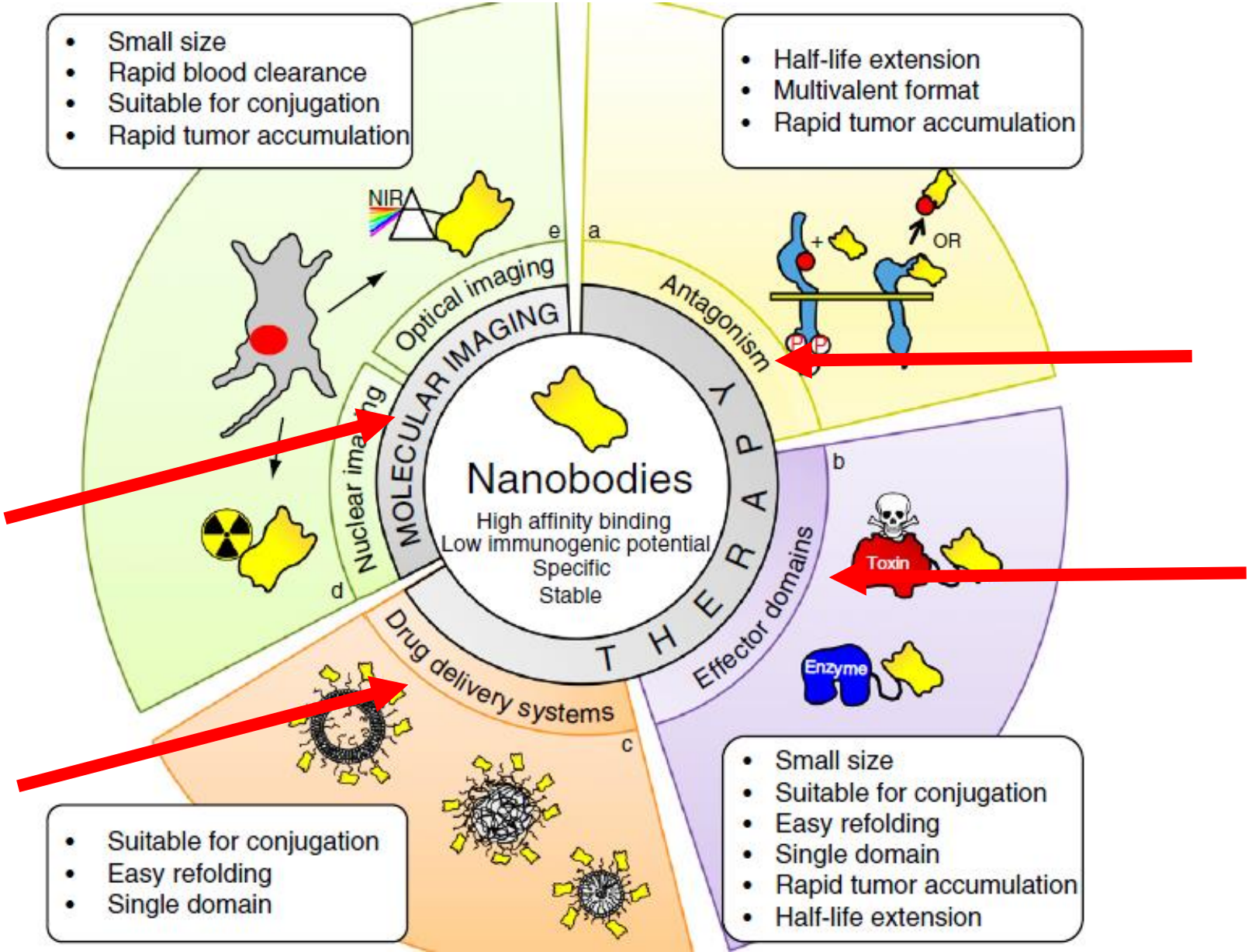
Nanobody Application

Disease Treatment
Dengue virus
H5N1 influenza
viral infection
Head & **Neck** Cancers
VEGF , HGF , **EGFR**
Venom Therapy
Aflatoxins In Agro-products
Thrombotic Thrombocytopenic **Purpura**
Plasmodium Knowlesi **Malaria** Vector



Heavy chain only antibodies

Nanobody Application



Clinical Drugs

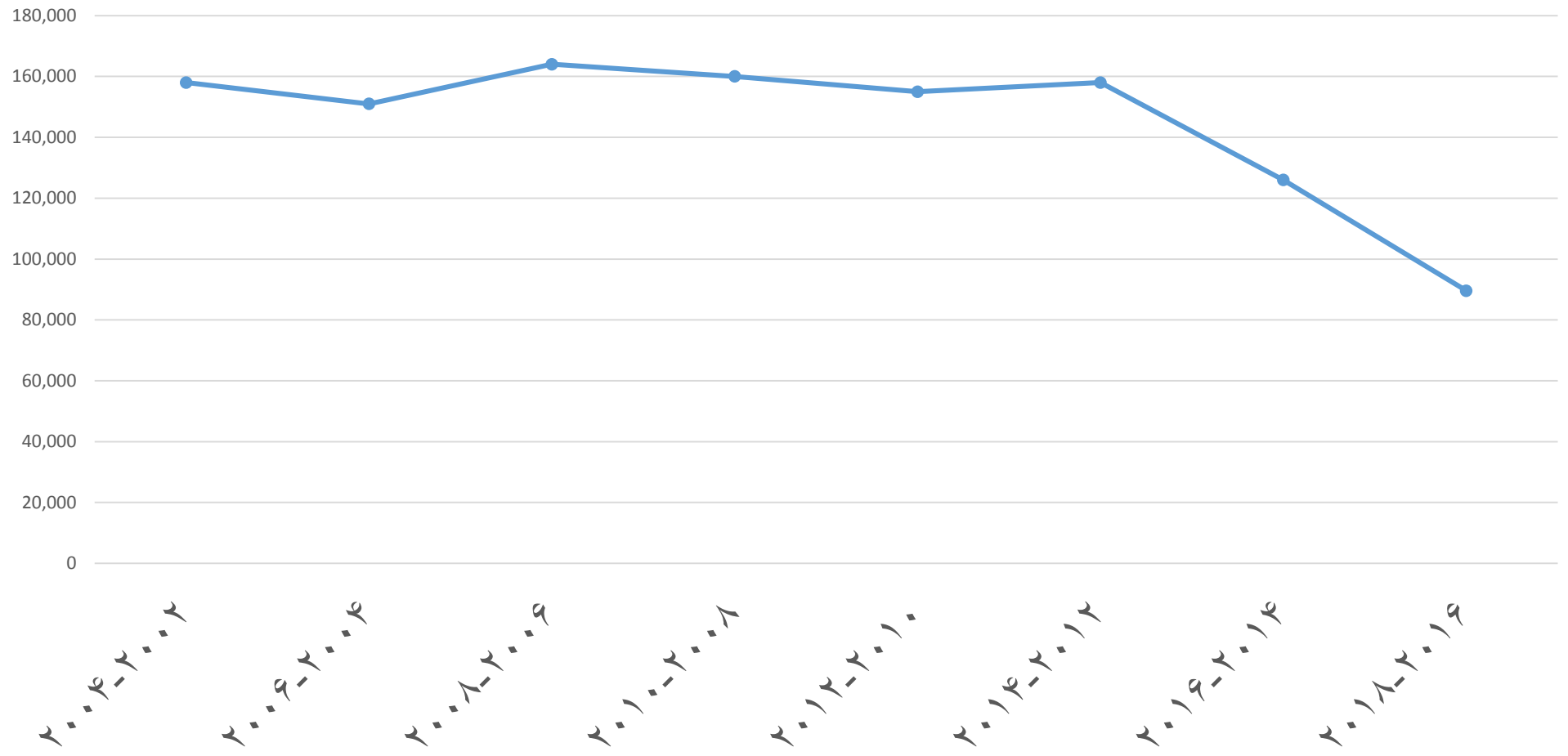
ATN-103 and PF-05230905	***Anti-TNFα*** *Nanobodies in* ***Rheumatoid Arthritis***
ALX-0081 and **ALX-0681**	***Anti-vWF*** *Nanobodies in* ***Haematology And Thrombotic Disorders***
ALX-**0141**	***Anti-RANKL*** *Nanobodies In Diseases Characterised By* ***Unwanted Bone Loss***
ALX-**0061**	***Anti-IL-6R*** *Nanobodies in* ***Rheumatoid Arthritis***
ALX-0651	***Anti-CXCR4*** *Nanobodies in* ***stem cell mobilisation***
ALX-0171	***Anti-RSV*** *Nanobodies in* ***Respiratory Viral***

Advantages & Disadvantages

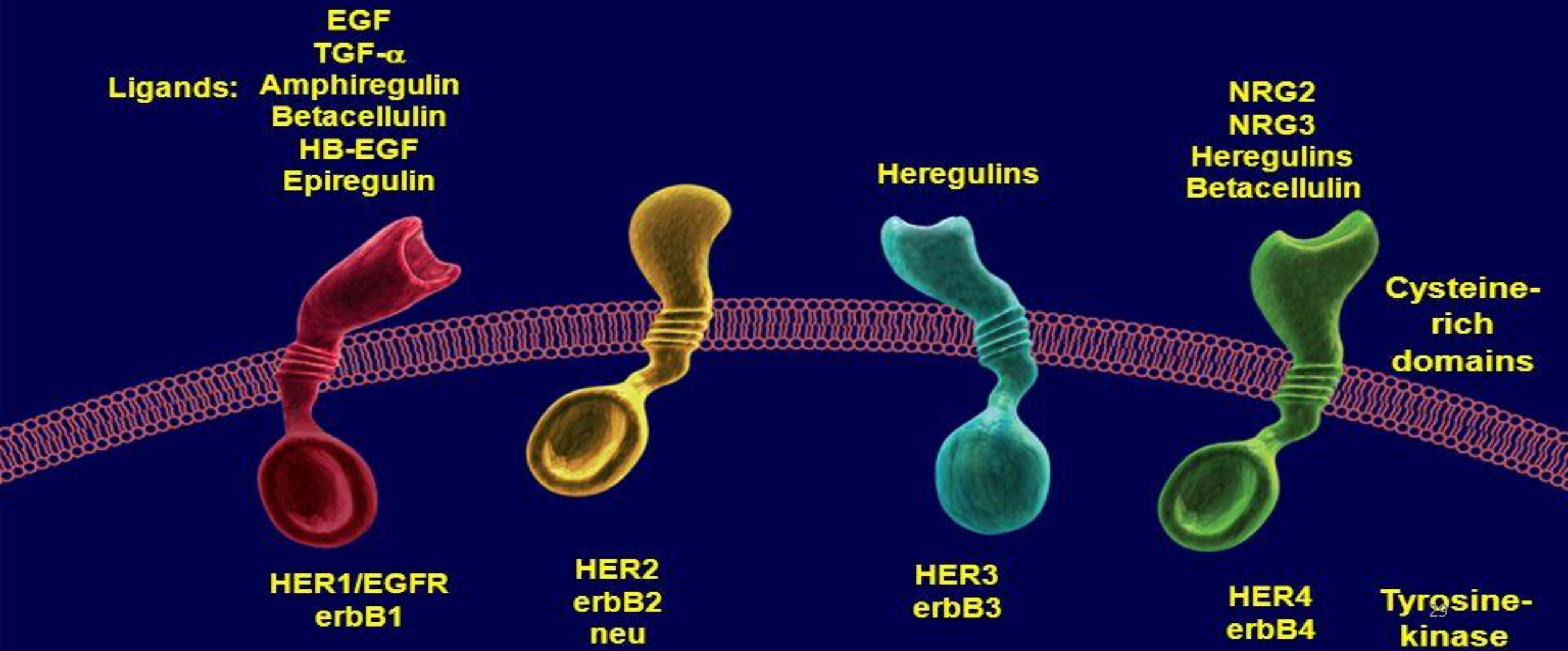
Advantages	Disadvantages
Accessibility	**Small Size (Fast Clearance)** Is Below The Renal Cut-off
Stability	
Solubility	
Engineerable	
1. Yields, Mg From Bacteria Grown In Simple Shake Flasks 2. Eukaryotic Cell Lines And Plants 3. Facile Production In Microbial Hosts	
1. Better Bio-distribution Into Tissues	

HER2

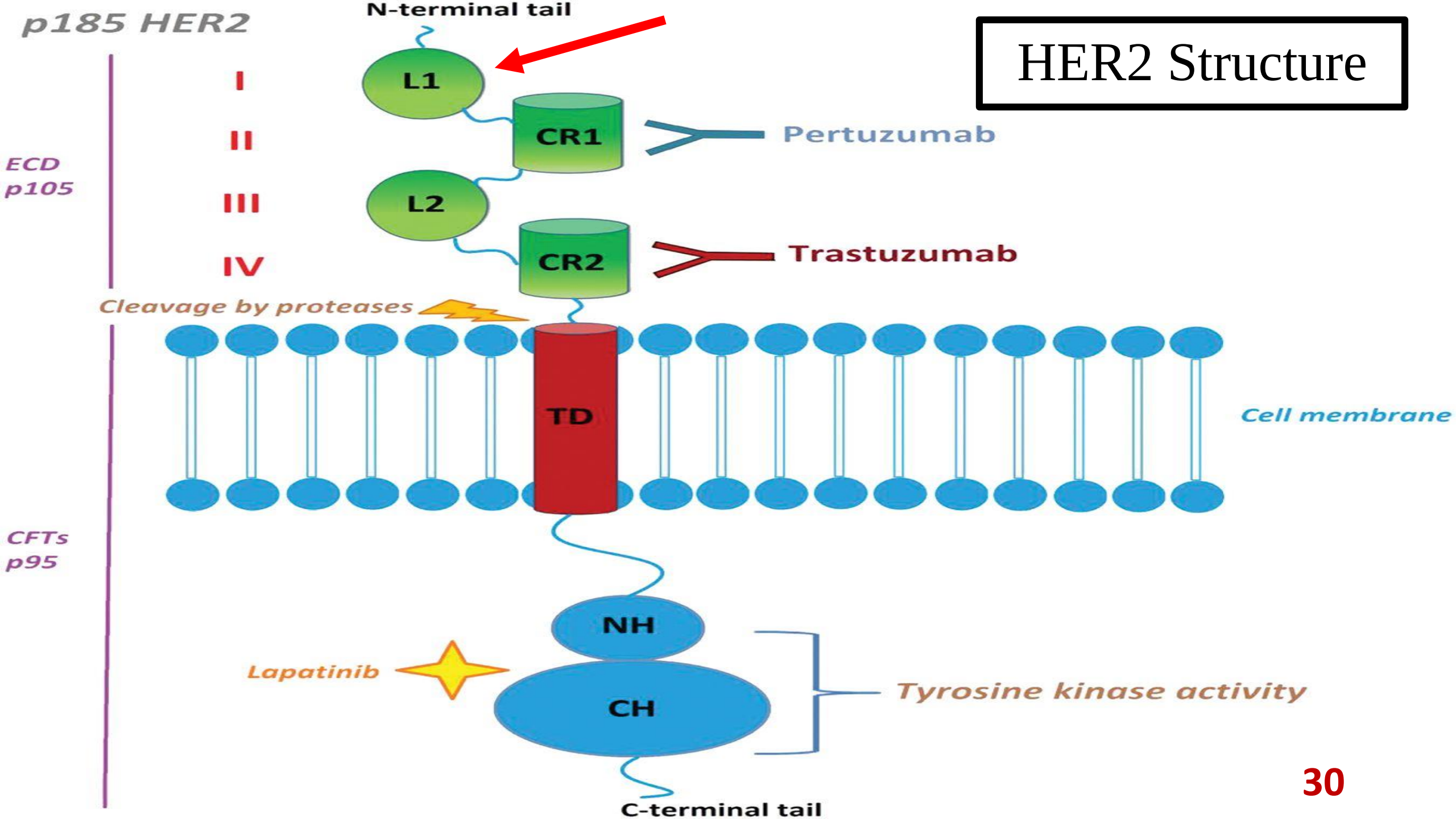
Research Trend Of HER2

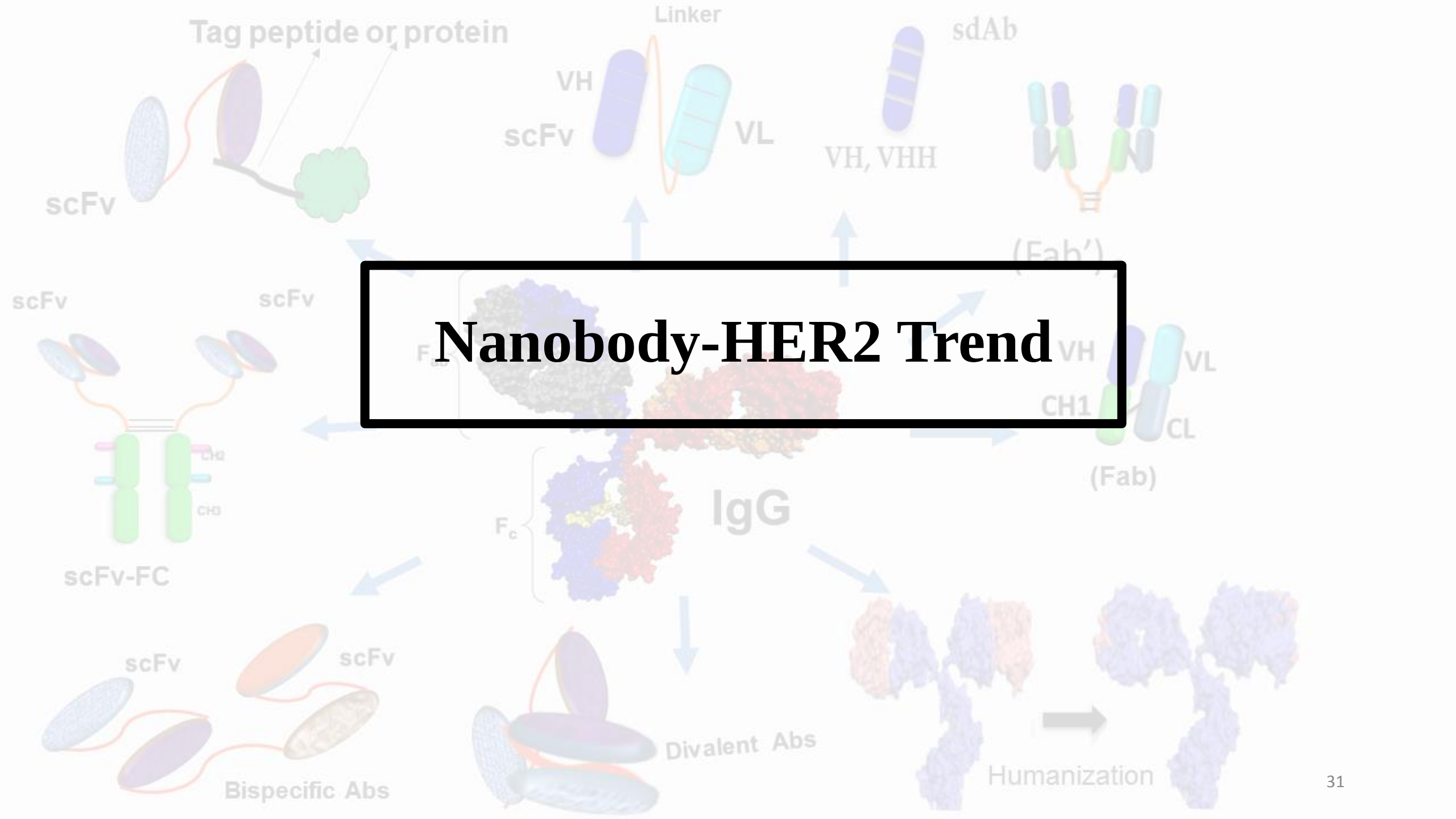


The HER Family of Receptors



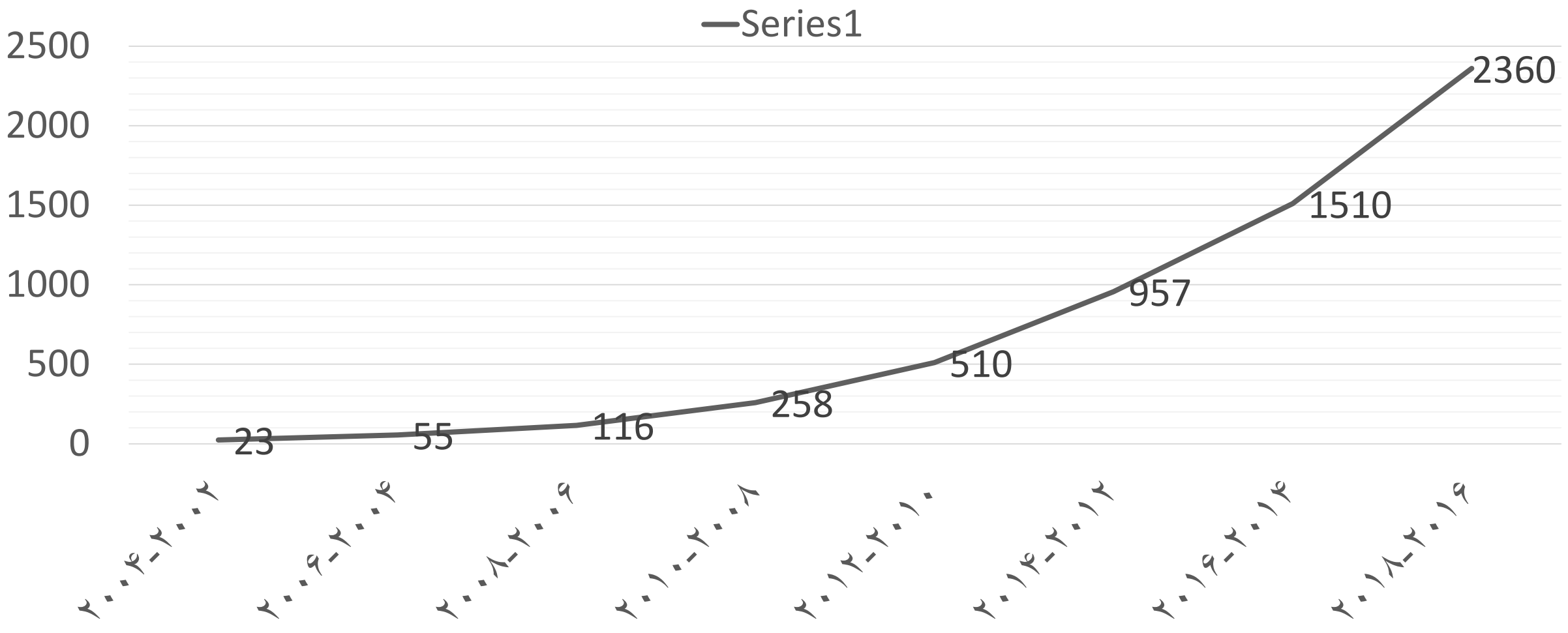
HER2 Structure





Nanobody-HER2 Trend

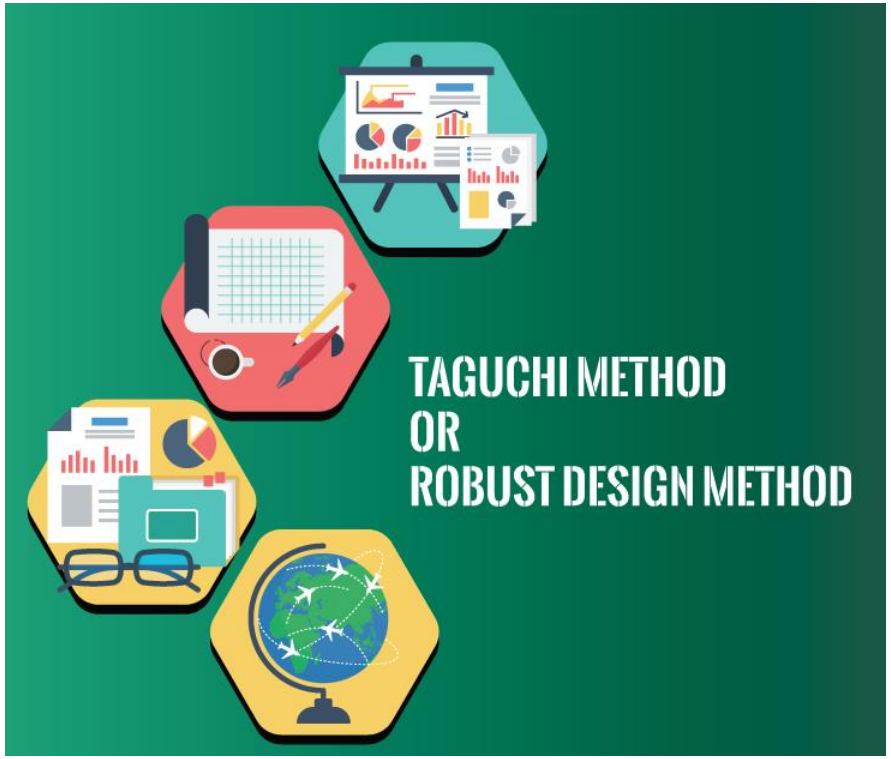
Research Of Nanobody-HER2 Trend



Taguchi Method

- Through This Method Of Experimental Design For Process Optimization
- Optimal Conditions For :

1. **Tem**perature
2. Inducer **Concentration**
3. **Induction** Period
4. Culture **Media**
5. **Type** Of Vector
6. Host **Strain**



Nanobody-HER2

- **131I-labeled sdAb :**
- Treat HER2-overexpressing cancer.
- Anti-HER2 sdAb 2Rs15d :
- Labeled with 131I using [131I]SGMIB and evaluated in vitro
- **The structure of the 2Rs15d-HER2 complex :**

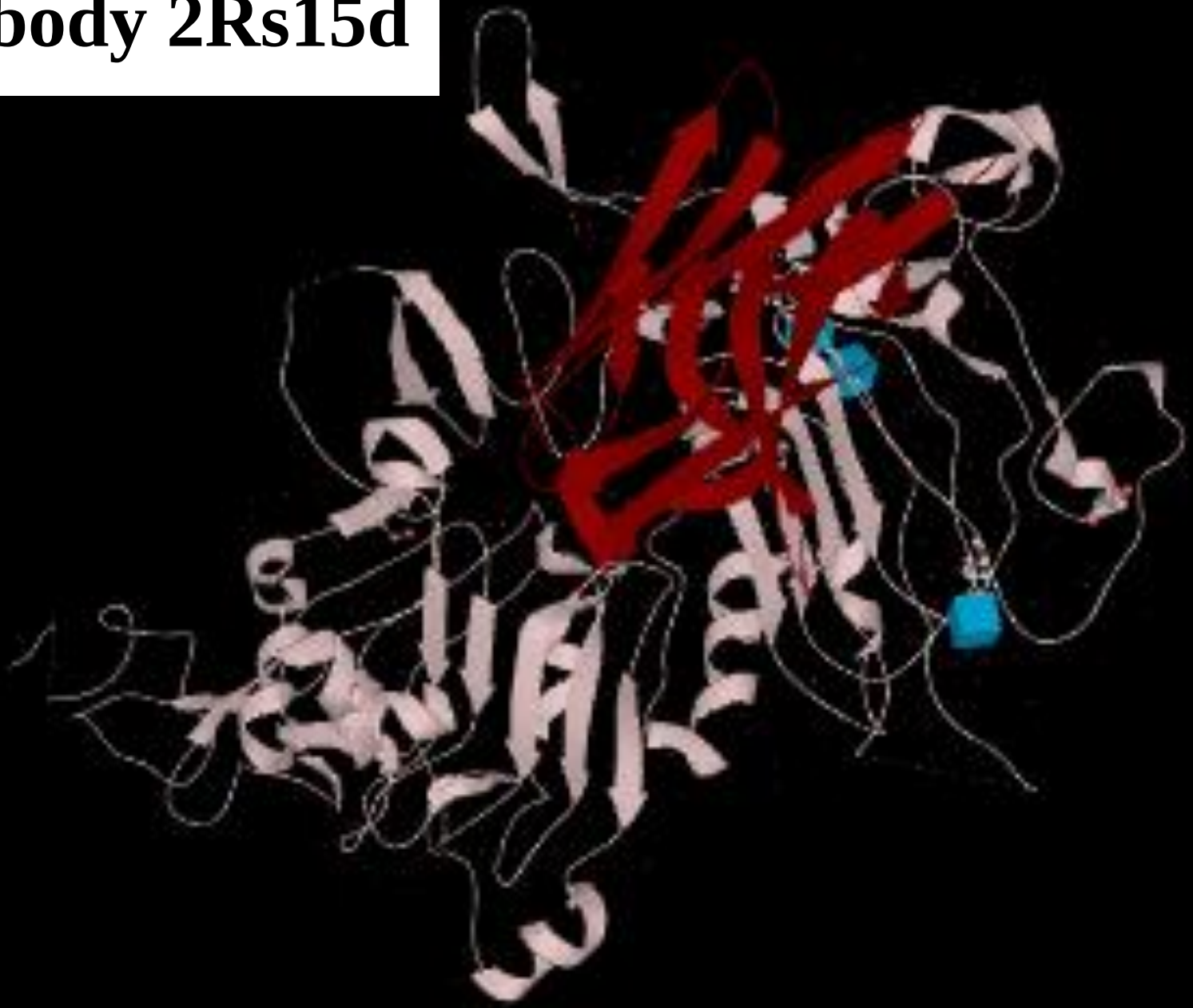
1. **X-ray** crystallography
2. Recognizes HER2 Domain 1
3. Bound specifically to HER2+ cells (**KD=4.74±0.39nM**)

- Success of future therapeutic sdAb :
- Reducing kidney retention of radiolabeled sdAbs
- anti-HER2 sdAb using the radiohalogen 131I
- 2Rs15d radiolabeled with 131I via the residualizing prosthetic group N-succinimidyl 4-Guanodinomethyl-3-[*I]benzoate ([*I]SGMIB)
- **[*I]SGMIB was twofold:**
- (i) rapidly clearing catabolites (reduce kidney dose of small radiolabeled biomolecules that are filtered via kidneys)
- (ii) the high pKa of its guanidino group interferes with the transport of labeled catabolites out of lysosomes, thereby trapping the radioiodine in cancer cells

Determination of the HER2-2Rs15d complex crystal structure

- Contribute to HER2 recognition interactions with HER2 are mediated by :
- **2Rs15d** residues located in the (**CDRs**)
- **Equal** number of **aa** located in (**FRs**)
- **Interactions** between **2Rs15d** and **HER2** domain **I** is presented in Supplementary Fig. S1D and Supplementary Table S2.
- Binding affinity, 0 to 300 nM

5my6 › Nanobody 2Rs15d



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THANK YOU